

ESCMID Study Group for Biofilms

Executive committee 2012: Chairman: Niels Høiby, Denmark (hoiby@hoibyniels.dk), Vice Chairman: Gianfranco Donelli, Italy, Secretary: Christine Imbert, France, Treasurer: Thomas Bjarnsholt, Denmark, Member: Antonio Oliver, Spain, Member: Johan van Eldere, Belgium, Coopted member (Eurobiofilms 2013): Tom Coenve, Belgium, Coopted member (Eurobiofilms 2015): Veronika Holà, (Czech Republic)

Bacterial biofilms are structured communities of bacterial cells enclosed in a self produced polymeric matrix, adherent to a surface. The majority of bacterial chronic infections are due to bacterial biofilms. Studies of biofilms have revealed differentiated, structured groups of cells with community properties. Understanding of the genetics and molecular basis may provide new therapeutic targets and means to control biofilm infections.

Biofilm structures

Biofilms constitute a protected mode of growth that allows survival in the hostile environment. The biofilm consists of microcolonies encapsulated by exopolysaccharide (EPS) produced by the bacteria (Figure 1- 4), but most of the biofilm is made of water channels which operate as a distribution system of nutrients and oxygen (Figure 5).

Figure 1
Gram-stained sputum from a 44 years old cystic fibrosis (CF) patient with mucoid and non-mucoid *P. aeruginosa* since 1970. A detached alveolus with a *P. aeruginosa* biofilm is seen (x25 & x100) (Høiby, 2004).

Figure 2
Confocal laser scanning microscopy (CLSM) image of Live/dead stained sputum sample from patient with CF (green cells are alive and red cells are dead) (J.A.J. Haagensen, 2005).

Figure 3
CLSM image of sputum sample from patient with CF after fluorescent in situ hybridization (FISH). A *P. aeruginosa* specific probe (red) identifies *P. aeruginosa* colony directly in the sputum sample (J.A.J. Haagensen and L. Yang, 2005).

Biofilms, inflammation and quorum-sensing

EPS, especially the polysaccharide alginate (mucoid phenotype) has an important role and function in CF. It acts as a scavenger of free oxygen radicals, prevents phagocytosis, and binds many antibiotics such as aminoglycosides (Figure 6).

P. aeruginosa controls the production and secretions of virulence factors by cell-to-cell communication signals in a process called quorum sensing (QS). The QS communication apparatus is composed of the *Las* and the *Rhl* systems. *P. aeruginosa* biofilms employs the QS signals to manipulate the host immune-response (Figure 7).

Figure 4A
Biliary stents with biofilm and amorphous material both on the inside and outside of the stent.

Figure 4B
Gram-stain (x1000) microscopic picture of the bacteria in the biofilm, Gram-negative rods and Gram-positive streptococci (Johansen et al, 2005).

Figure 5
CLSM images of various structures of 7 days old flow-cell biofilms of Gfp tagged *P. aeruginosa* from chronically infected CF patients (Lee et al., 2005).

Figure 6
Gram-stained sputum from a CF patient. *P. aeruginosa* biofilm surrounded by PMNs (Høiby, 2004).

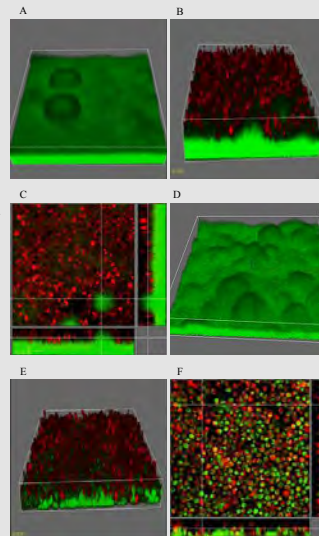


Figure 7
PMNs and non-mucoid *P. aeruginosa* biofilm in vitro – (CLSM pictures)
A) Wild-type PAO1 biofilm (gfp tagged). B) 2.5 h with PMNs stained with SYTO62 (PMN appear red fluorescent). C) Cross-section showing the wild-type biofilm with PMNs on top. D) $\Delta lasR-rhlR$ PAO1 mutant. E) 3-D projection and F) Cross-section showing the $\Delta lasR-rhlR$ mutant fully penetrated by PMNs and the disappearance of much of the biomass (Bjarnsholt, T, Jensen, P.Ø. et al., Microbiology, 151,373-83, 2005).

Biofilms and antibiotics

Biofilm bacteria are generally more tolerant to antibiotic treatment than their planktonic bacteria counterpart. Antibiotic doses which kill suspended cells, for example, need to be increased as much as 1,000 x to kill biofilm cells (Figure 8). Biofilms evade antimicrobial challenges by multiple mechanisms. These have been grouped into three broad categories:

- 1) reduction of the antimicrobial concentration in the bulk fluid surrounding the biofilm (Figure 9).
- 2) failure of the antimicrobial agent to penetrate the biofilm; and
- 3) adoption of a resistant physiological state or phenotype by at least a fraction of the cells in the biofilm.

Figure 8
Biofilm protects the bacteria from the antibiotic treatment. Induction of β -lactamase in biofilm treated with ceftazidime (CLSM pictures). PAO1 with PampC-gfp (ASV): 6 days old biofilm exposed to 100 μ g/ml ceftazidime (125 X MIC) for 4 h. Gfp expression indicates expression of AmpC β -lactamase in the biofilm. Detection level of the monitor: 10 μ g/ml ceftazidime (Bagge et al., Antimicrob Agents Chemother. 2004; 48:1168–74).

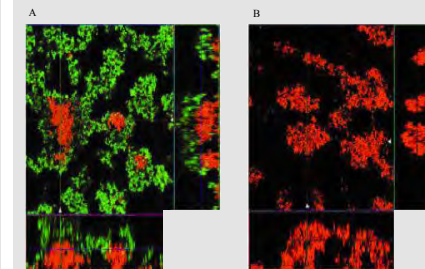


Figure 9
PAO1 with PampC-gfp (ASV): 6 days old biofilm exposed to 0.5 μ g/ml imipenem (1/80 MIC) for 4 hours (A) and uninduced biofilm (B). Gfp expression indicates expression of AmpC β -lactamase in the biofilm. (Detection level of the monitor ≥ 0.1 μ g/ml imipenem) (Bagge et al., Antimicrob Agents Chemother. 2004;48:1168–74).

ESCMID STUDY GROUP FOR BIOFILMS (ESGB) – founded April 2005 during 15th ECCMID in Copenhagen - will organize the following activities:

- 1) Propose an annual ESGB workshop to be held in conjunction with the annual ECCMID.
- 2) Suggest an annual biofilm symposium during the ordinary scientific programme of the annual ECCMID.
- 3) Organize an annual biofilm workshop between the annual ECCMIDs consisting of invited lectures and a poster session.
- 4) Establish a ESGB website with a ESGB Newsletter with information about the activities of the study group. This informations will also be communicated to all members of ESCMID in ESCMID NEWS.
- 5) During the annual workshops, educational activities including hands-on technical education will be planned and organized if technical possible.
- 6) Suggest guidelines for prophylaxis, diagnosis and therapy of biofilm infections.
- 7) An active cooperation with the ASM biofilm meetings will be proposed.

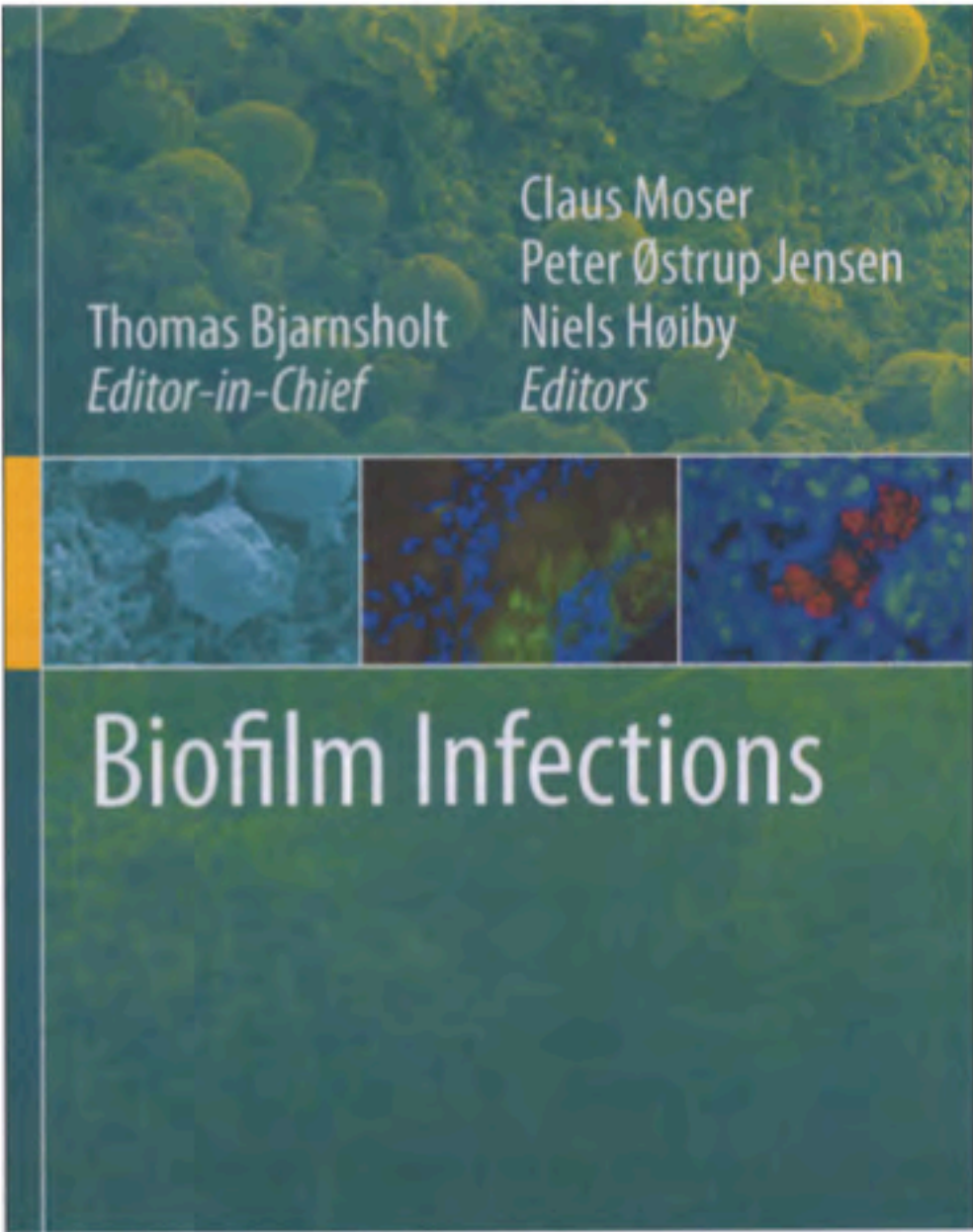
- **ESGB performance 2005-2011:**
- **ESBG 2005-ESCMID-ECCMID: Key-note lectures/2hr & 1hr symposia/educational workshops, 2006-11**
- **ASM-Biofilmconference Quebec 2007**
- **BAMBHAR/Mallorca 2007**
- **DTU, Lyngby, Technical Course 2009, 2010**
- **EUROBIOFILMS 2009 Rome**
- **Biofilms in nosocomial fungal infections Paris 2011**
- **Book: Biofilm Infections, Springer, 2010**
- **EUROBIOFILMS 2011 Copenhagen**
- **On-line course in biofilm : www.biofilmcourse.ku.dk, University of Copenhagen 2011**
- **ESGB future plans:**
- **ESBG 2005-ESCMID-ECCMID: Key-note lectures/2hr & 1hr symposia/educational workshops, 2012 etc.**
- **DTU, Lyngby, Technical Course 2012**
- **Consensus conference: Diagnostic and treatment of biofilm infections 2012 together with ESGIAI**
- **EUROBIOFILMS 2013, 2015 etc.**

ESGB 2011 report

2011 has been a successful year for our Study Group. A new book 'Biofilm Infections' (pringer) was published by T. Bjarnsholt, C. Moser, P.Ø. Jensen & N. Høiby (eds) with 17 chapters by 52 international authors on all aspects of medical biofilms and biofilm techniques. Then followed a highly successful ESCMID postgraduate education course "Biofilm in nosocomial fungal infections" organized at Institut Pasteur on January 31st to February 1st 2011 which were attended by approximately 130 participants. The chair organizers, Christine Imbert and Anne Beauvais have to be congratulated for their very successful efforts. In July 2011 we had the Eurobiofilms 2011 in Copenhagen on July 5th to 8th 2011 which was the second European biofilm meeting which we have organized (the first one was in Rome 2009) and again the meeting was big success with more than 300 participants from 31 countries from 6 continents listening to 6 key note lectures, 13 symposia, 66 oral presentations (40 also presented as posters) and studying additional 164 posters. The day before the congress we organized 4 workshops with 58 participants. A special issue of FEMSIM is being published with articles from Eurobiofilms 2011. Thomas Bjarnsholt, treasurer of ESGB also organized a new on-line course in Biofilms in the autumn 2011 through University of Copenhagen(www.biofilmcourse.dk) which can also be found on WWW.escmid.org/ESGB. We have also in 2011 decided that the Eurobiofilms 2013 will take place in September 2013 in Gent, Belgium, organized by Tom Coenye, Johan van Eldere and Patrick van Dijck and the Eurobiofilms 2015 will take place in Brno, Czech Republic organized by Veronika Holá. Additionally we will organize the third ESGB technical course "Medical Biofilms Techniques" at DTU on August 27th to 30th 2012. We are also in the planning stage of organizing a consensus and guidelines meeting on diagnosis and treatment of medical biofilms together with two other study groups of ESCMID (Study Group of Nosocomial Infections and Study Group for Implant-Associated Infections).

Niels Høiby

On behalf of the board of ESGB

The cover of the journal 'Biofilm Infections' features a dark green background with a subtle, textured pattern of circular, cell-like structures. In the upper right, the names of the editors are listed in white text. Below the names, there is a horizontal strip containing three small, square images: the first is a light blue micrograph of a cell, the second is a dark blue micrograph with many small, bright spots, and the third is a blue micrograph with red, irregular shapes. The title 'Biofilm Infections' is printed in large, white, sans-serif font across the middle of the cover. The Springer logo and the issue information are at the bottom.

Thomas Bjarnsholt
Editor-in-Chief

Claus Moser
Peter Østrup Jensen
Niels Høiby
Editors

Biofilm Infections

Springer November 2010

We are repeating the success

Based on the great evaluation and success of the first course during the fall of 2011 we are now opening for applications for the 2012 online course on "Bacterial Biofilms and Their Role in Chronic Diseases". The course can be followed by both Master degree and PhD students, as well as professionals that live up to the admission criteria. For registration and more information, please visit:

www.biofilmcourse.ku.dk

Application deadline is 1 June 2012 (MSc- and prof.)

Application deadline is 6 August 2012 (PhD students)



Teachers at the course:

Director J. William Costerton

Centre for Genomic Sciences, Allegheny-Singer Research Institute, USA

Assoc Prof Marvin Whiteley

Section of Molecular Genetics and Microbiology, The University of Texas at Austin, USA

Prof Matt Parsek

Department of Microbiology, University of Washington, USA

Director, Phil Stewart

Chemical and Biological Engineering, Montana State University, USA

Prof, Niels Højby, MD, Claus Ernst Moser and Dr, Peter Østrup Jensen

Department of Clinical Microbiology, Copenhagen University Hospital, Denmark

Luanne Hall-Stoodley

Southampton Wellcome Trust Clinical Research Facility and University of Southampton Respiratory BRU, Southampton General Hospital, UK

Assoc Prof, Thomas Bjarnsholt, Prof Michael Givskov and Assoc Prof Tim Tolker-Nielsen

Faculty of Health Sciences, University of Copenhagen, Denmark

Prof, Søren Molin

Department for Systems Biology, Technical University of Denmark

2nd FUNGAL BIOFILMS MEETING

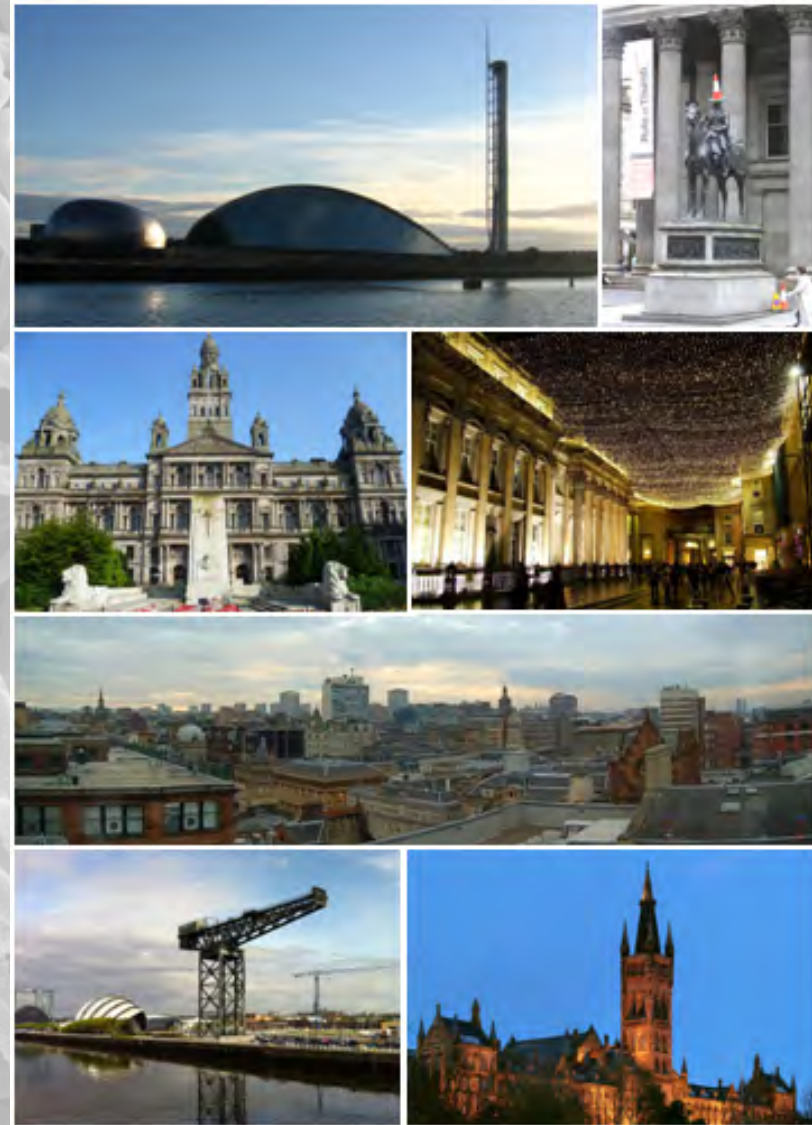
GLASGOW (UK), MAY 20th & 21st 2013

Local Organizers

Prof Craig Williams
Department of Microbiology
Royal Hospital for Sick Children
Dalnair Street
Glasgow, G3 8SJ
Phone +44 141 201 0422
Craig.Williams@ggc.scot.nhs.uk



Dr Gordon Ramage
Glasgow Dental School
School of Medicine
University of Glasgow
378 Sauchiehall Street
Glasgow, G2 3JZ
Phone +44 141 211 9752
gordon.ramage@glasgow.ac.uk



Organising Committee

Prof Craig Williams
Dr Gordon Ramage
Dr Anne Beauvais
Dr Christine Imbert
Prof Niels Hoiby



The Trades Hall
of GLASGOW

<http://www.tradeshallglasgow.co.uk/>

Preliminary Sessions

Clinical implications of fungal biofilms
Pathogenesis and Inflammation
Polymicrobial biofilms
Biofilm technologies
Antifungal resistance
Molecular aspects of fungal biofilms

Prices (2 days, lunch, refreshments, reception)

Professional £150 (£100 per day) Student £50

Confirmed Speakers

Dr David Andes (Madison, USA)
Dr Anne Beauvais (Paris, France)
Prof Geraldine Butler (Dublin, Ireland)
Dr Tom Coenye (Ghent, Belgium)
Prof Marcel Gutierrez-Correa (Lima, Peru)
Dr Mariana Henriques (Braga, Portugal)
Dr Christine Imbert (Poitiers, France)
Prof Howard Jenkinson (Bristol, UK)
Prof Aaron Mitchell (Pittsburgh, USA)
Dr John Morrissey (Cork, Ireland)
Dr Carol Munro (Aberdeen, UK)
Dr Gordon Ramage (Glasgow, UK)
Dr Riina Rautemaa-Richardson (Manchester, UK)
Prof Jose Lopez Ribot (San Antonio, USA)
Prof Craig Williams (Glasgow, UK)
Dr David Williams (Cardiff, UK)

EUROBIOFILMS 2013

Third European Congress on Microbial Biofilms Basic and Clinical Aspects

GHENT, BELGIUM, SEPTEMBER 9-12, 2013

www.eurobiofilms2013.ics.dk

Organising Committee: Tom Coenye (president), Patrick Van Dijk (vice-president, basic), Johan Van Eldere (vice-president, clinical) and the ESGB Executive Committee

Scientific Advisory Board: Niels Hoiby, Gianfranco Donelli, Christine Imbert, Thomas Bjarnsholt, Craig Williams, Antonio Oliver, and Veronika Hola. More to be confirmed.

Conference Venue

Ghent University Aula, Voldersstraat, Ghent, Belgium



Pre-conference workshops: Pre-conference workshops will be organised on 9 September 2013. Topics of the workshops will include "Identification of biofilm-associated micro-organisms" and "Animal models for studying biofilms". More topics will be announced in the near future.

Main Sessions will take place from 9 September (evening) until 12 September (lunch time). Topics that will be addressed include mixed-species biofilms, microbial fuel cells, biofilm-related chronic wound infections, signalling in biofilms, clinical and basic aspects of fungal biofilms, mechanisms of biofilm resistance & tolerance, medical-device related biofilm infections, oral biofilms, biofilm heterogeneity & evolution, and microbial biofilm ecology.



Abstract Submission

You are kindly invited to submit abstracts describing research work that you wish to present at the Congress, either orally or as a poster. Deadline for abstracts for poster and oral sessions: March 15th, 2013. Guidelines for submission will be available on the congress website in the near future.

Registration & Hotel Accommodation

Information on the registration and hotel booking will be available on the conference website.

Congress Secretariat:

International Conference Services A/S
Copenhagen, Denmark,
Mail: eurobiofilms2013@ics.dk

Scientific Secretariat:

Laboratory of Pharmaceutical Microbiology
Ghent University, Ghent, Belgium
Mail: eurobiofilms2013@ugent.be

Supported by European Society of Clinical Microbiology and Infectious Diseases (ESCMID) / www.escmid.org



www.eurobiofilms2011.ics.dk

hoiby@hoibyniels.dk

ESCMID Study Group for Biofilms

www.escmid.org/esgb

Website: <http://www.esgb.eu>

EUROBIOFILMS 2011
Second European Congress on Microbial Biofilms –
Basic and Clinical Aspects

COPENHAGEN, DENMARK, JULY 6-8, 2011

Organising Committee: Niels Høiby (president), Søren Molin (vice president, basic), Gianfranco Donelli (vicepresident, clinical), Thomas Bjørnsholt (secretary) and the ESGB board

Scientific Advisory Board: Alejandra Bosch, Oana Ciofu, Gianfranco Donelli, Johan van Eldere, Alain Filloux, Hans-Curt Flemming, Michael Givskov, Christine Imbert, Peter Østrup Jensen, Helle Krogh Johansen, Staffan Kjelleberg, Per Klemm, Michael Kühl, Paolo Landini, Claus Moser, Per H. Nielsen, Bente Nyvad, Marco Oggioni, Antonio Oliver, Robert Palmer, Matt Parsek, Holger Rohde, Claus Sternberg, Philip Stewart, Paul Stoodley, Søren Sørensen, Kazuhiro Tateda, Tim Tolker-Nielsen, Paul Williams, Chen Yiqiang

Conference Venue
Panum Institute, University of Copenhagen, DK

Target Audience

This multi disciplinary conference target clinical microbiologists, infectious disease doctors, dentists, veterinarians, basic scientists working within the biofilm area and students on all levels.

Abstract Submission

You are kindly invited to submit abstracts describing research work that you wish to present at the Congress, either orally or as a poster. Abstracts must be submitted no later than 1 March 2011. Please consult the congress website for guidelines for submission www.eurobiofilms2011.ics.dk.

Registration & Hotel Accommodation

Information on the registration and hotel booking can be found on the website www.eurobiofilms2011.ics.dk where you can register online or download the registration and hotel form.

Registration Fee

The registration fee includes the scientific sessions, printed congress material, lunches and coffee breaks, but not travel and accommodation costs (see congress website www.eurobiofilms2011.ics.dk)

CME Accreditation

The organizer of the congress will apply for European CME accreditation



Precongress Workshops: Animal models of biofilms, Microfluidic systems and confocal microscopy, Molecular diagnostics of biofilm bacteria, New techniques for visualization of biofilms, Micro-sensors

Main Sessions: 'Diagnosis of biofilm infections', 'Regulation of biofilm development', 'Treatment of biofilm infections', 'Airway and wound biofilm infections and host response', 'Novel methodology for studies of biofilm structure and composition and omics', 'Dental biofilms', 'Diversification and evolution in biofilms', 'Biofilm-based medical device related infections and host response', 'Biofilm community ecology', 'Resistance and tolerance of biofilms to antibiotics and host response', 'Fungal biofilms', 'Social interactions in Biofilms' 6 key-note lectures, poster session.

Deadline for abstracts for poster and oral sessions: March 1st, 2011.

Scientific Secretariat:

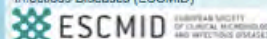
Thomas Bjørnsholt, Department of Clinical Microbiology 9301, Rigshospitalet, Copenhagen, Denmark.
Mail: tbjornsholt@sund.ku.dk

Congress Secretariat:



International Conference Services A/S, Copenhagen, Denmark,
Mail: eurobiofilms2011@ics.dk
Web: www.eurobiofilms2011.ics.dk

Endorsed and supported by
European Society of Clinical Microbiology and Infectious Diseases (ESCMID)



Danish Society of Clinical Microbiology
DANISH SOCIETY FOR
CLINICAL MICROBIOLOGY

www.eurobiofilms2011.ics.dk

THEMATIC ISSUE

FEMS

888

IMMUNOLOGY & MEDICAL MICROBIOLOGY

Biofilms II

Volume 65

Issue 2

July 2012

ISSN 0928-8244

Guest Editors:

Thomas Bjarnsholt

Gianfranco Donelli

Åke Försberg

Niels Høiby

Christine Imbert



Discover this journal online at
WILEY ONLINE LIBRARY
wileyonlinelibrary.com/journal/fems

FEMS

IMMUNOLOGY & MEDICAL
MICROBIOLOGY

Volume 65
Issue 2
July 2012

- Editorial**
125 Understanding biofilms – are we there yet?
T. Bjarnsholt, N. Høiby, G. Donelli, C. Imbert and Å. Försberg
- MiniReviews**
127 Towards diagnostic guidelines for biofilm-associated infections
L. Hall-Stoodley, P. Stoodley, S. Kishu, N. Høiby, C. Moser, J. William Costerton, A. Moser and T. Bjarnsholt
146 Combating biofilms
L. Yang, Y. Liu, H. Wu, Z. Song, N. Høiby, S. Molin and M. Givskov
158 Pathogenesis and treatment concepts of orthopaedic biofilm infections
W. Zimmerli and C. Moser
169 *Staphylococcus carnosus* – a model to uncover molecular mechanisms for yeast biofilm biology
R.K. Bajaj, K.S. Andersen and B. Regenberg
183 The interconnection between biofilm formation and horizontal gene transfer
J.S. Mathsen, M. Burnside, L.H. Hansen and S.J. Sørensen
196 Update on infectious risks associated with dental unit waterlines
V. Barbot, A. Robert, M.-H. Rodier and C. Imbert
205 Exploring the applications of invertebrate host-pathogen models for *in vivo* biofilm infections
S. Edwards and B.V. Kjellerup
215 *Pseudomonas* selected during chronic lung infection in cystic fibrosis patients: implications for the treatment of *Pseudomonas aeruginosa* biofilm infections
O. Ciffo, L.F. Mandberg, H. Wang and N. Høiby
- Research Articles**
226 Can Simpson's paradox explain co-operation in *Pseudomonas aeruginosa* biofilms?
A.S. Penn, T.C.R. Conibear, R.A. Watson, A.R. Krasovec and J.S. Webb
236 The microorganisms in chronically infected end-stage and non-end-stage cystic fibrosis patients
V.B. Rudky, T.R. Thomsen, M. Albede, K.N. Krug, P.H. Nielsen, U.R. Johansen, M. Givskov, N. Høiby and T. Bjarnsholt
245 The metabolically active subpopulation in *Pseudomonas aeruginosa* biofilms survives exposure to membrane-targeting antimicrobials via distinct molecular mechanisms
W.-C. Chang, S.J. Pamp, M. Nilsson, M. Givskov and T. Tolker-Nielsen
257 Elaboration of antibiotic surfaces functionalized with antifungal-cyclodextrin inclusion complexes
A. Gherli, V. Humbert, F. Turpin, C.-M. Pradier, C. Imbert and J.-M. Benjoud
270 Biofilm and planktonic *Enterococcus faecalis* elicit different responses from host phagocytes *in vitro*
K. Daw, A.S. Baghdady, S. Awasthi and N. Shankar
283 Kinetics and morphology of polymicrobial biofilm formation on polypropylene mesh
P. Stoodley, S. Sifhu, L. Hansen, M. Albede, A. Bous, L. Hall-Stoodley and S. Kishu
291 Bacterial diversity in suspected prosthetic joint infections: an exploratory study using 16S rRNA gene analysis
Y. Xu, V.B. Rudky, O. Simonsen, C. Pedersen, J. Lorenzen, H.C. Schanley, P.H. Nielsen and T.R. Thomsen
305 Catheter lock technique *in vitro* efficacy of ethanol for eradication of methicillin-resistant staphylococcal biofilm compared with other agents
A. Choudhury, J. Kargineni and V.B.
309 Direct analysis of bacterial viability in endotracheal tube biofilm from a pig model of methicillin-resistant *Staphylococcus aureus* pneumonia following antimicrobial therapy
I. Fernández-Barral, G. Li Bassi, M. Ferrer, A. Bosch, M. Calvo, J. Vilà, A. Górriz, P. Martínez-Olondriz, M. Rigol, M. Esperetti, N. Luque and A. Torres
318 Biofilm-growing intracellular anaerobic bacteria
G. Donelli, C. Kuete, B. Cardines and P. Macrassano
326 Thiophenones inhibit *Staphylococcus epidermidis* biofilm formation at nontoxic concentrations
J. Lönn-Stensrud, A.-O. Naemi, T. Bernheide, F.C. Petersen and A.A. Scheie
335 Combination of microscopic techniques reveals a comprehensive visual impression of biofilm structure and composition
M. Albede, A. Øvstrup, R. Lohre, N. Høiby, M. Givskov and T. Bjarnsholt
343 Virulence factors in *Proteus* bacteria from biofilm communities of catheter-associated urinary tract infections
V. Holo, T. Perutkova and F. Ruzicki
350 Biofilm formation of *Klebsiella pneumoniae* on urethral catheters requires either type 1 or type 3 fimbriae
S.G. Isaihu, C. Sörve, K.A. Kragh and A. Rasmussen
360 Biofilm building capacity of *Salmonella enterica* strains from the poultry farm environment
E. Schoneveld, L.L. Nesse, R. Hauck, D. Windhorst, H.M. Høfz and L.K. Vestby
366 Polysaccharides serve as scaffold of biofilms formed by mucoid *Pseudomonas aeruginosa*
L. Yang, W. Hengshuang, H. Wu, S. Damkjaer, N. Jochimsen, Z. Song, M. Givskov, N. Høiby and S. Molin
- Short Communications**
377 The role of biofilm matrix polysaccharide Psl in mucoid *Pseudomonas aeruginosa* biofilms
I. Ali, S. Wang, D. Wang, M.R. Parak and D.J. Wozniak
381 Application of atmospheric pressure nonthermal plasma for the *in vitro* eradication of bacterial biofilms
M.T. Alkawarek, Q.T. Alqawi, S.P. Gorman, W.G. Graham, D. O'Connell and B.F. Gilmore
385 Considering hydrophobicity as a bacterial biofilm disease
S. Kishu, L.-M. Joshi and P. Stoodley
390 Identification and characterization of 4-[4-(3-phenyl-2-propen-1-yl)-1-piperazinyl]-5H-pyrimido[5,4-b]indole derivatives as *Salmonella* biofilm inhibitors
S.C.A. Rodrigues, B. De Pauw, B. Leusen, A. Marchand, P. Chotin, S.C.J. De Keersmaecker, J. Vanderleyden and H.P.L. Steenackers
395 Synergistic phage-antibiotic combinations for the control of *Escherichia coli* biofilms *in vitro*
E.M. Ryan, M.T. Alkawarek, R.F. Connolly and B.F. Gilmore



Information on this journal (including Abstracting and Indexing information) can be accessed at:
wileyonlinelibrary.com/journal/fems
This journal is available online at Wiley Online Library. Visit wileyonlinelibrary.com to search the articles and register for table of contents and e-mail alerts.

30 Biofilm Articles authored by speakers at EUROBIOFILMS 2011, Copenhagen, Denmark, July 2011. Dedicated to J.W. Costerton who died May 2012.

We would like to thank the West China School of Stomatology biofilm conference team and the *IJOS* editorial team for their hard work in preparation of this special conference and *IJOS* issue. We also wish to thank all sponsors for their generous support.



Keynote Speakers in the International Symposium of Microbial Biofilm
Chengdu China April 6th-8th, 2011

International Journal of Oral Science

(Founded in 2009)

Online submission via:
<http://www.ijos.org.cn>

Sponsored by:
Sichuan University

Volume 3 Number 2 April 2011

Contents

PREFACE

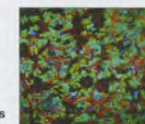
- 47 Preface for the microbial biofilm issue
Wen-yuan Shi, Xue-dong Zhou

REVIEWS

- 49 Multispecies communities: interspecies interactions influence growth on saliva as sole nutritional source
Paul E. Kolenbrander
- 55 The clinical impact of bacterial biofilms
Niels Heiby, Oana Clofu, Helle Krogh Johansen, Zhi-jun Song, Claus Moser, Peter Ørstap Jensen, Søren Molin, Michael Givskov, Tim Tøtler-Nielsen, Thomas Bjørnskov
- 66 The role of bacterial biofilm in persistent infections and control strategies
Li Chen, Yu-mei Wen
- 74 Current understanding of multi-species biofilms
Liang Yang, Yang Liu, Hong Wu, Niels Heiby, Søren Molin, Zhi-jun Song

ORIGINAL ARTICLES

- 82 Oxygen dependent pyruvate oxidase expression and production in *Streptococcus sanguinis*
Lan-yan Zheng, Andreas Iizek, Zhi-yun Chen, Jens Kreth
- 90 Analysis of interspecies adherence of oral bacteria using a membrane binding assay coupled with polymerase chain reaction-denaturing gradient gel electrophoresis profiling
Ren-ke Wang, Xue-song He, Wei Hu, Renate Lux, Ji-yao Li, Xue-dong Zhou, Wen-yuan Shi
- 98 Influences of trans-trans farnesol, a membrane-targeting sesquiterpenoid, on *Streptococcus mutans* physiology and survival within mixed-species oral biofilms
Jao-Gyu Jeon, Santosh Pandit, Jin Xiao, Stacy Gregoire, Megan L. Falsetta, Marilee I. Klein, Hyun Koo



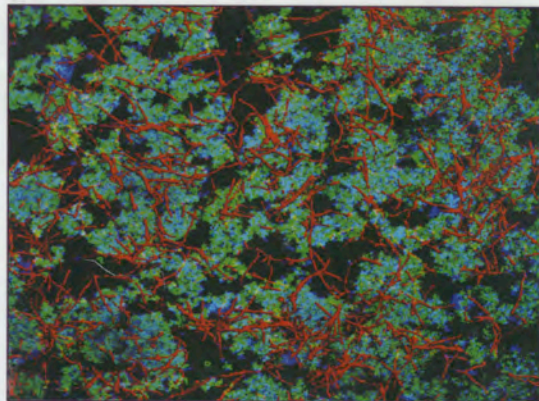
Cover: Representative confocal micrograph of a three-genera biofilm grown in a flow cell using saliva as the sole nutritional source for growth. See page 51 by Paul E. Kolenbrander for detail.

Coordinating Editor for this issue
Xing-rang Hu

First Chinese international Biofilm Conference, Chengdu, PR China, April 2011

微生物生物膜与感染

Microbial Biofilm and Infection



主 编 周学东 施文元

人民卫生出版社

微生物生物膜与感染

Microbial Biofilm and Infection

名誉主编 闻玉梅 Bill Costerton Neils Høiby

主 编 周学东 施文元

编 委 (以姓氏笔画为序)

马旅雁	王人可	王世伟	亓庆国	刘 东
许 涛	孙桂芹	李 蒙	李 燕	李继遥
杨 亮	杨 蕾	杨维青	杨霁舟	肖丽英
肖晓蓉	吴 红	吴 旻	余加林	宋 超
宋志军	张 平	张真印	陈 力	武 侠
武有聪	周学东	胡 涛	施文元	秦 毅
贾文祥	徐 欣	彭宁宁	董 莹	谢 轶
薛 晶	瞿 滌			

主编助理 贾文祥

人民卫生出版社

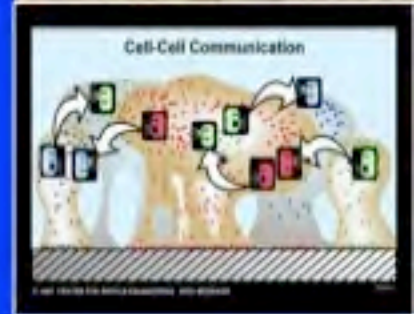
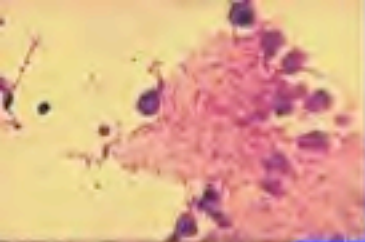
First Chinese textbook about Biofilms, published 2012

What is a biofilm?

- Appearance - how does it look
- Where is it located/persists: Surface: - body - outer/inner
Surface: - artificial
Inside body
- Physiology
- Biochemistry
- Genetics
- Resistance: To innate and adaptive defence mechanisms
To antibiotics/disinfectants
- Pathology - pathogenesis - disease

Definition: A structured consortium of bacterial cells surrounded by a self-produced polymer matrix, mono/polyspecies

BIOFILM INFECTION = Chronic infection = an infection which A) persists in spite of therapy, and in spite of the host's immune- and inflammatory response, and B) is characterized by persisting pathology and immune response (in contrast to colonization)



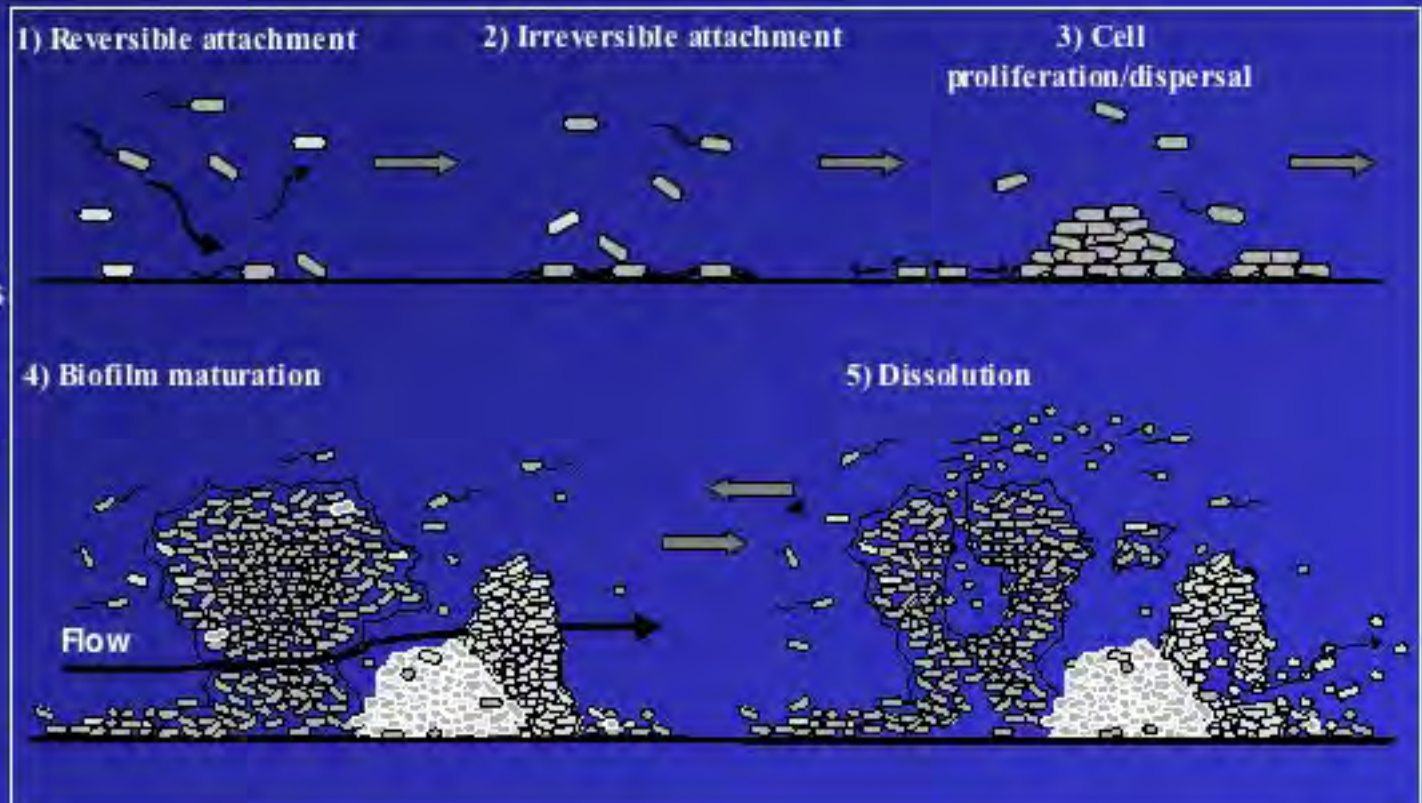
Gradients

CASE

Complex Adaptive Systems Ecology

Biofilm development – consensus?

In hydrodynamic conditions biofilm development depends on **adhesive forces**. At the substratum bacterial **motility** may have significant impacts on structure development.



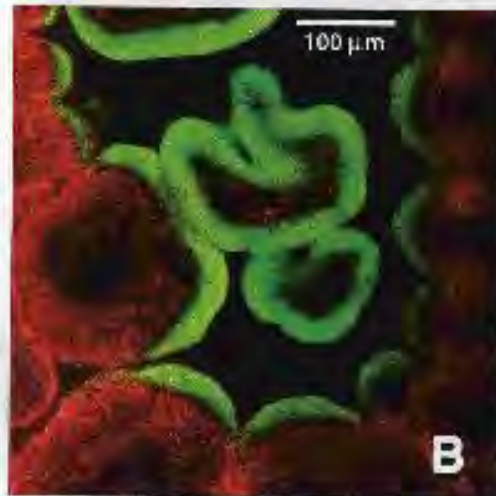


FIG. 3. Scattered pattern of GFP expression in *P. aeruginosa* biofilms grown in glass capillary tubes under continuous flow conditions. Strain PAO1(pAD1) was grown for 24 h and then induced with IPTG for 4 h. Panel A shows a laser transmission view, and panel B shows a fluorescence image of the same spot. Green areas are due to GFP and red areas are due to the chloramphenicol resistance.



FIG. 4. Stratified patterns of GFP expression in frozen sections of *P. aeruginosa* colony biofilms. Green areas are due to GFP and red areas are due to the chloramphenicol resistance. Panel A shows a regular control in which a colony biofilm formed by strain PAO1(pAD1) was the substrate. Panel B shows a biofilm of the same strain after 4 h of induction with IPTG. Panel C shows colony biofilm formed by the reporter strain AD298.

Microbiol.
(Werner et al.
Stratified
growth in *P.*
aeruginosa
biofilms.
Appl.
Environment
70:6188-96;
2004)

Red: control
stain of
biofilm
bacteria

Green = GFP
= metabolic
active
bacteria at
the biofilm
surface

Some general features of biofilm infections in humans compared to acute planktonic infections and superficial colonization/normal flora on skin and mucosal membranes. **The bold fonts indicate biofilm specific features**

Features of biofilm infections	Necessary condition for biofilm infections	Sufficient condition for biofilm infections	Also found in acute planktonic infections	Also found in colonization/normal flora on skin and mucosal membranes
Aggregates of bacteria embedded in a self-produced polymer matrix	Yes	Yes	No	No/Yes
Tolerant to clinical relevant PK/PD dosing of antibiotics in spite of susceptibility of planktonic cells	Yes	Yes	No	No/Yes
Tolerant to both innate and adaptive immune response	Yes	Yes	No	No/Yes - unknown (s-IgA)
Inflammation	Yes	No	Yes	No
Biofilm-specific antigens	No and Yes - seldom – e.g. <i>Pseudomonas aeruginosa</i> alginate	No and Yes - seldom – e.g. <i>Pseudomonas aeruginosa</i> alginate	No	No

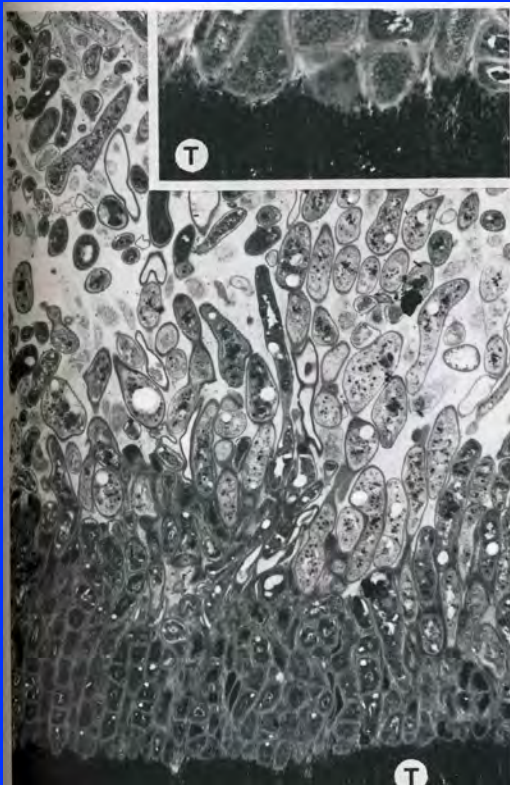
(Høiby et al.:Antibiotic resistance of bacterial biofilms Internat. J. Antimicrob. Agents 35:322-32; 2010)

Some general features of biofilm infections in humans compared to acute planktonic infections and superficial colonization/normal flora on skin and mucosal membranes. **The bold fonts indicate biofilm specific features**

Features of biofilm infections	Necessary condition for biofilm infections	Sufficient condition for biofilm infections	Also found in acute planktonic infections	Also found in colonization/normal flora on skin and mucosal membranes
Antibody response	Yes - after some weeks	No	Yes - after some weeks	No
Chronic infections	Yes	Yes	No	No
Foreign body associated infections	No	Yes	No but yes the first day of infection	No
Located on surfaces	No	No	Yes	Yes
Localized infection	Yes	No	Yes	Yes
Focus for spreading or local exacerbation	Yes	No	Yes	Yes

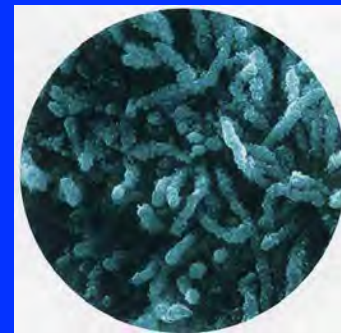


**S. epidermi-
dis biofilm
on an i.v.
device. SEM**



Dental biofilm,
subgingival plaque
(Fiehn & Larsen: Oral
bacteriology. In: Høiby
(ed.) Basic and Clinical
Microbiology. FADL,
Copenhagen, 1998)

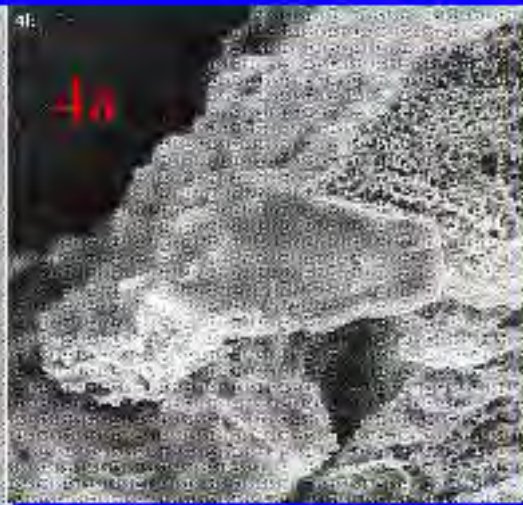
CARIES & PERIODONTITIS



DENTAL PLAQUE IS A BIOFILM.
Evidence points to at least six
bacterial species acting in a
consortium that can lead to
periodontitis, although no single
species has been linked to the
disease in all cases.

(Costerton & Stewart:
Battling biofilms.
Emerging Trends in
Oral care. Scientific
American Jan. 2002)

HØIBY 2011



Stickler, D.: Urinary catheters: ideal sites for the development of biofilm communities. Microbiology today 32:22-25; 2005

3: SEM of a biofilm on a patient's encrusted catheter. *E.coli*, *E. faecalis* & *P. mirabilis* were found in this biofilm community.

4 a-c: Worm-like structure that blocked a patient's catheter at 4 days interval. *E. faecalis*, *P. aeruginosa*, *E. coli*, *P. mirabilis* were recovered from the biofilm. The patient produced 15 of these worms over a 10-week period

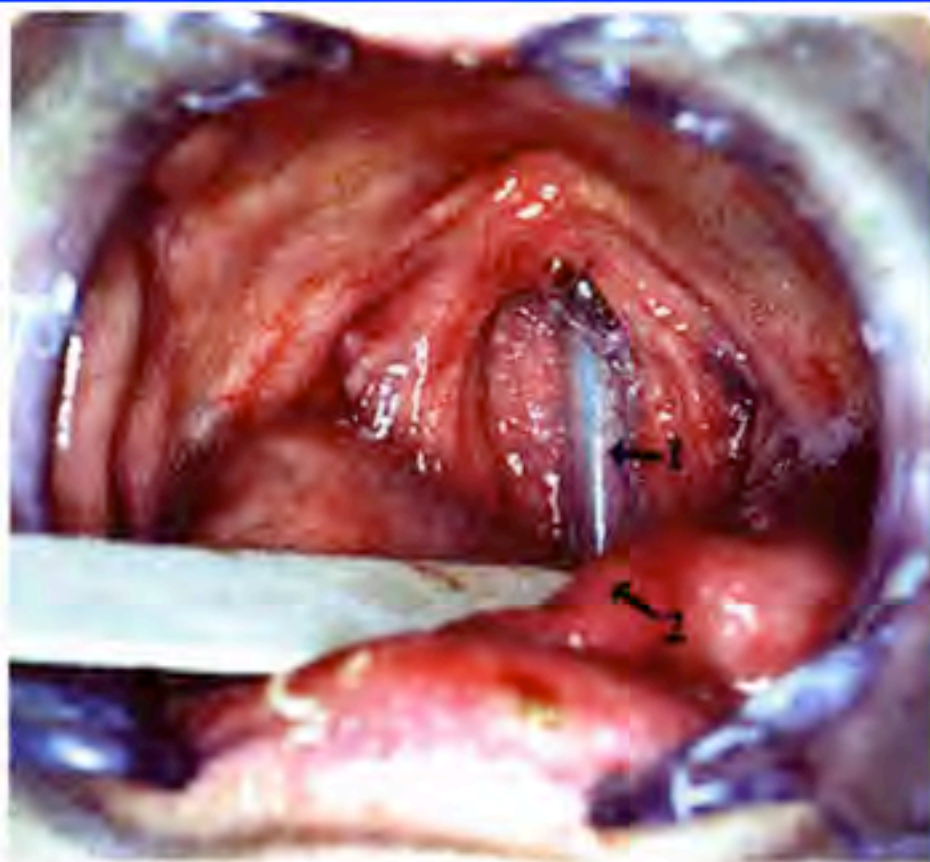


Figure 1. Nasogastric tube embedded in the nasopharynx. 1, nasogastric tube; 2, dorsum of tongue.

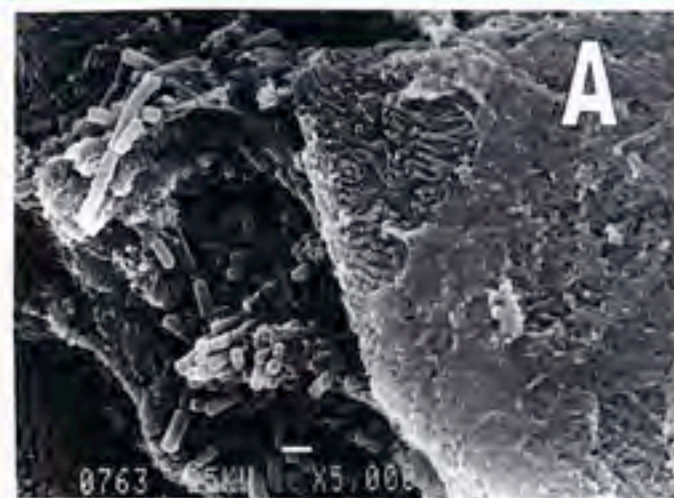


Figure 2. Representative biofilms on nasogastric tubes showing bacterial organisms with typical form of *Pseudomonas aeruginosa*. Scanning electronic microscope. A, scale bar, 1 μ m; B, scale bar, 10 μ m.

BIOFILM ON NASOGASTRIC TUBES (Leibovitz et al.: *P. aeruginosa* and the oropharyngeal ecosystem of tube-fed patients. *Emerg. Infect. Dis.* 9:956-59; 2003)

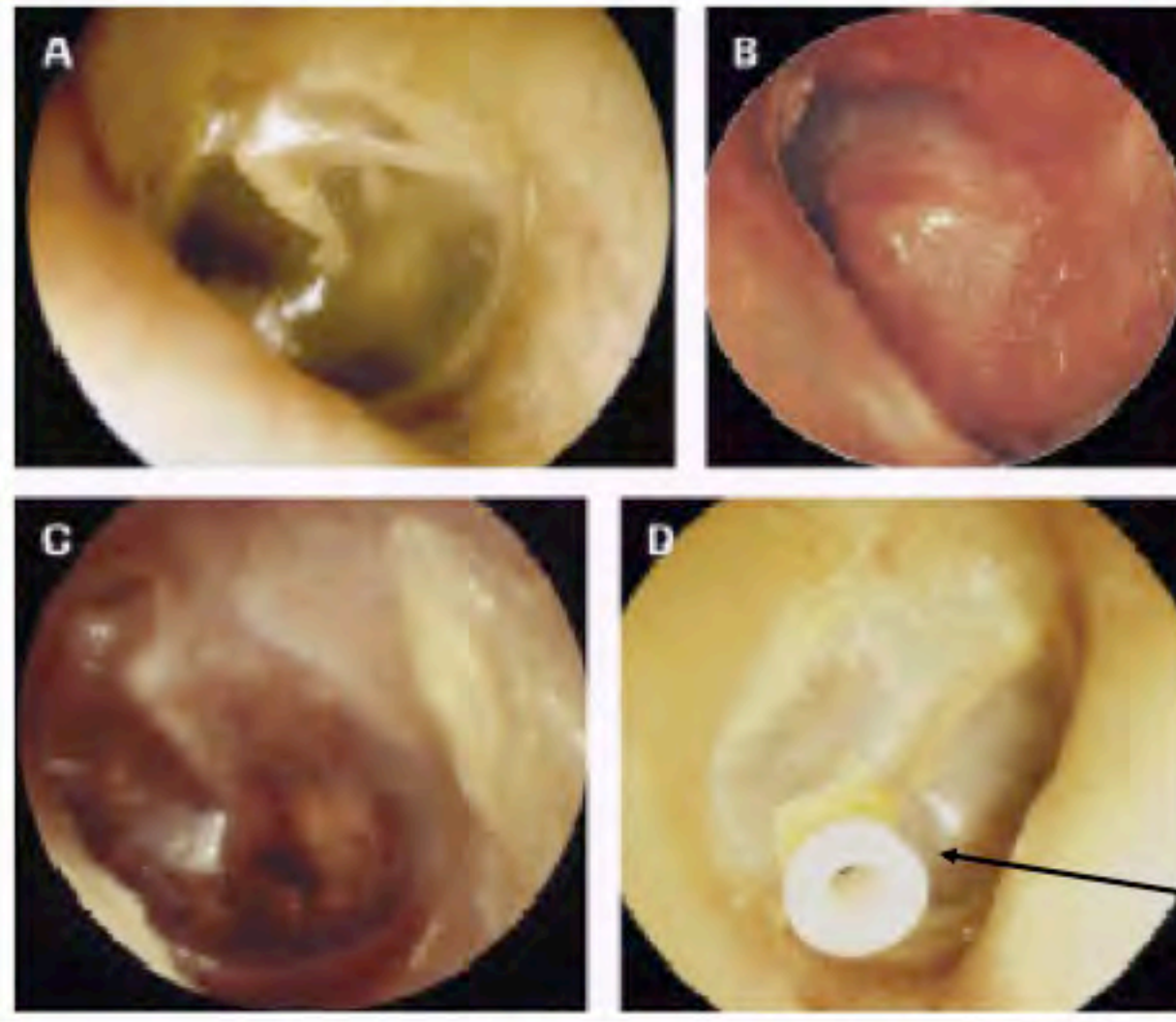


(Inglis: Evidence for Dynamic Phenomena in Residual Tracheal Tube Biofilm. Br J Anaesth 1993;70:22-24)

VENTILLATOR ASSOCIATED PNEUMONIA

BIOFILM ON VENTILLATION TUBES

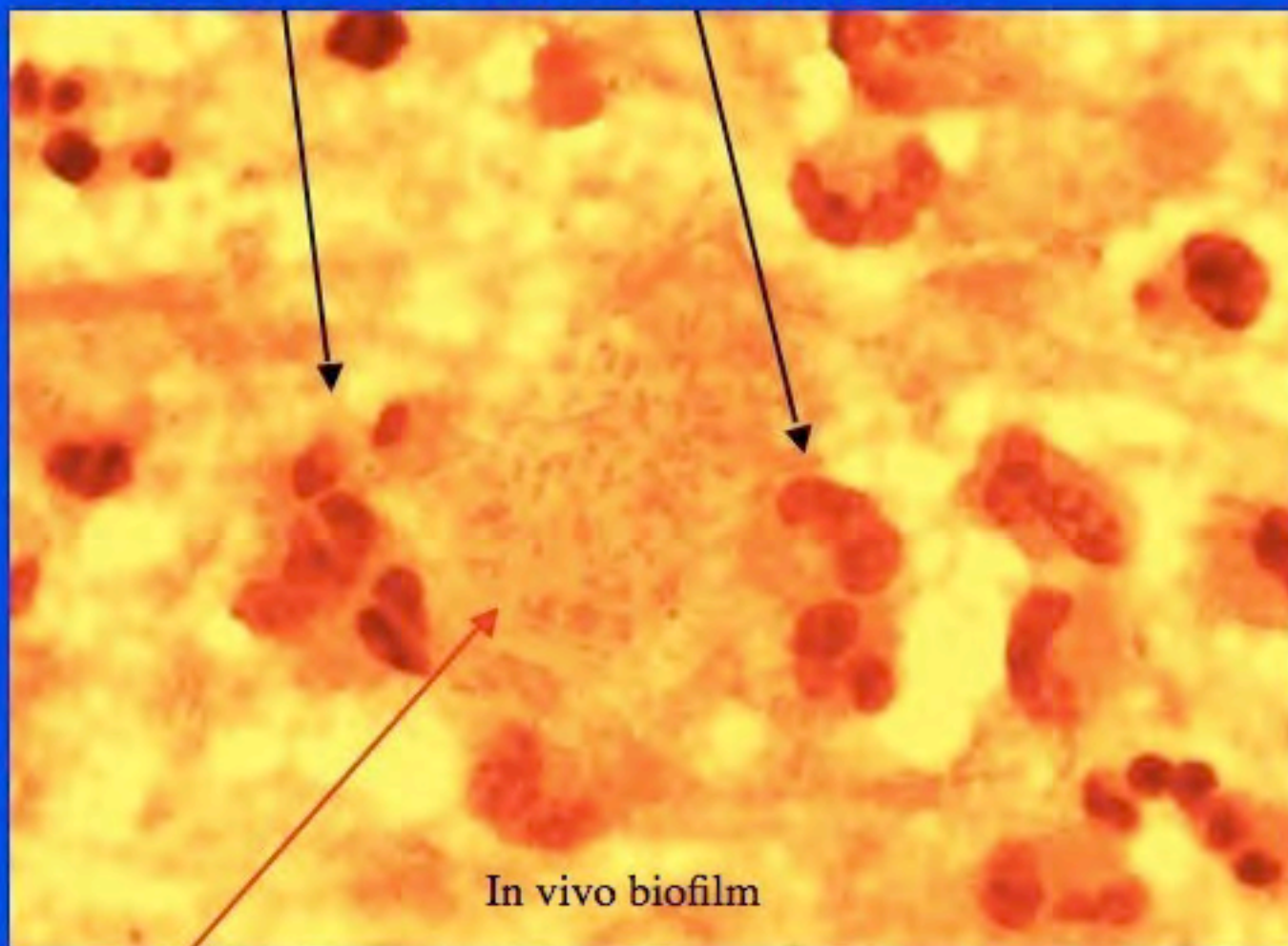
A) Normal middle ear, B) acute otitis media, C) Otitis media wwith effusion, D) Ventillation tube



Biofilm!

(Rovers et al. Otitis Media. Lancet 363:465-73;2004)

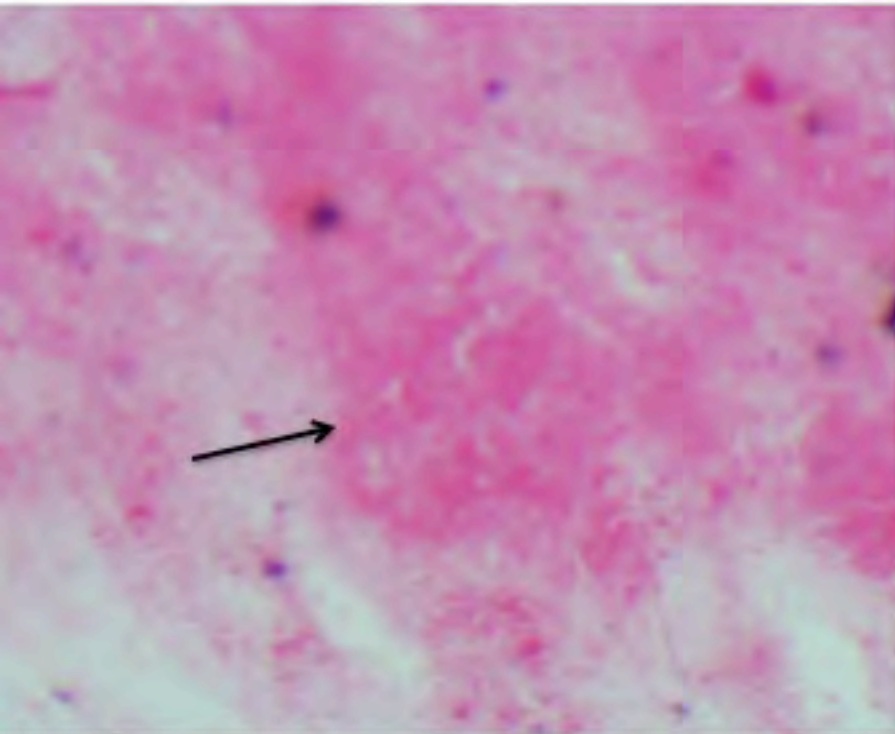
POLYMORPHONUCLEAR LEUKOCYTES



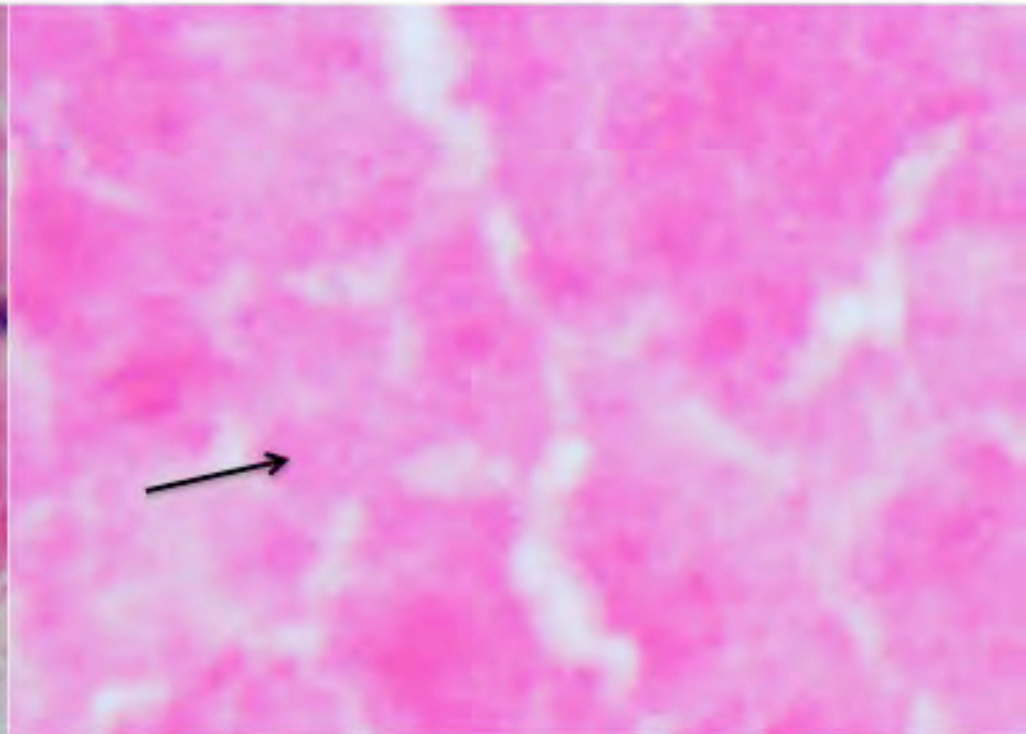
CF sputum - biofilm: **DIVERSITY!**

HØIBY 2004

Biofilm in sputum of 14 CF patients with chronic *A. xylosoxidans* (7/8) or *B. multivorans* (6/6) lung infection



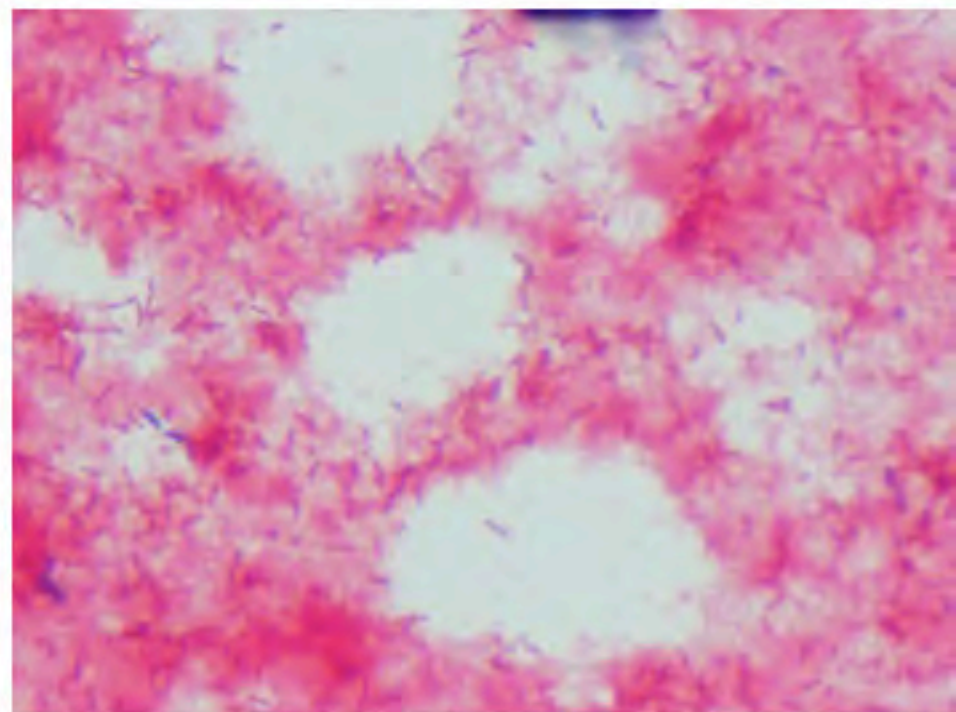
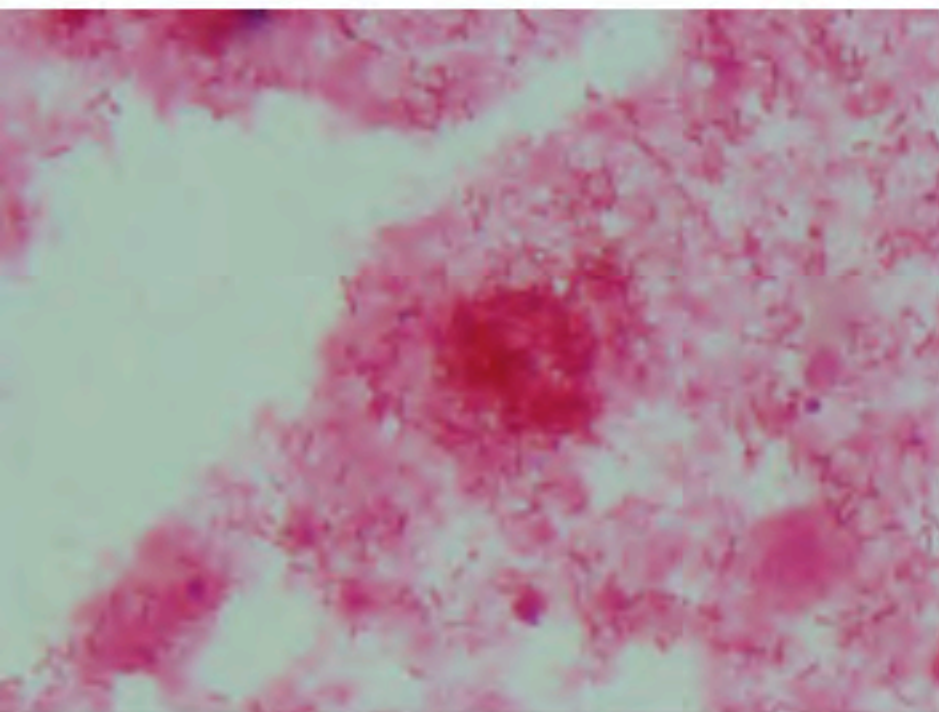
Gram stain x 40



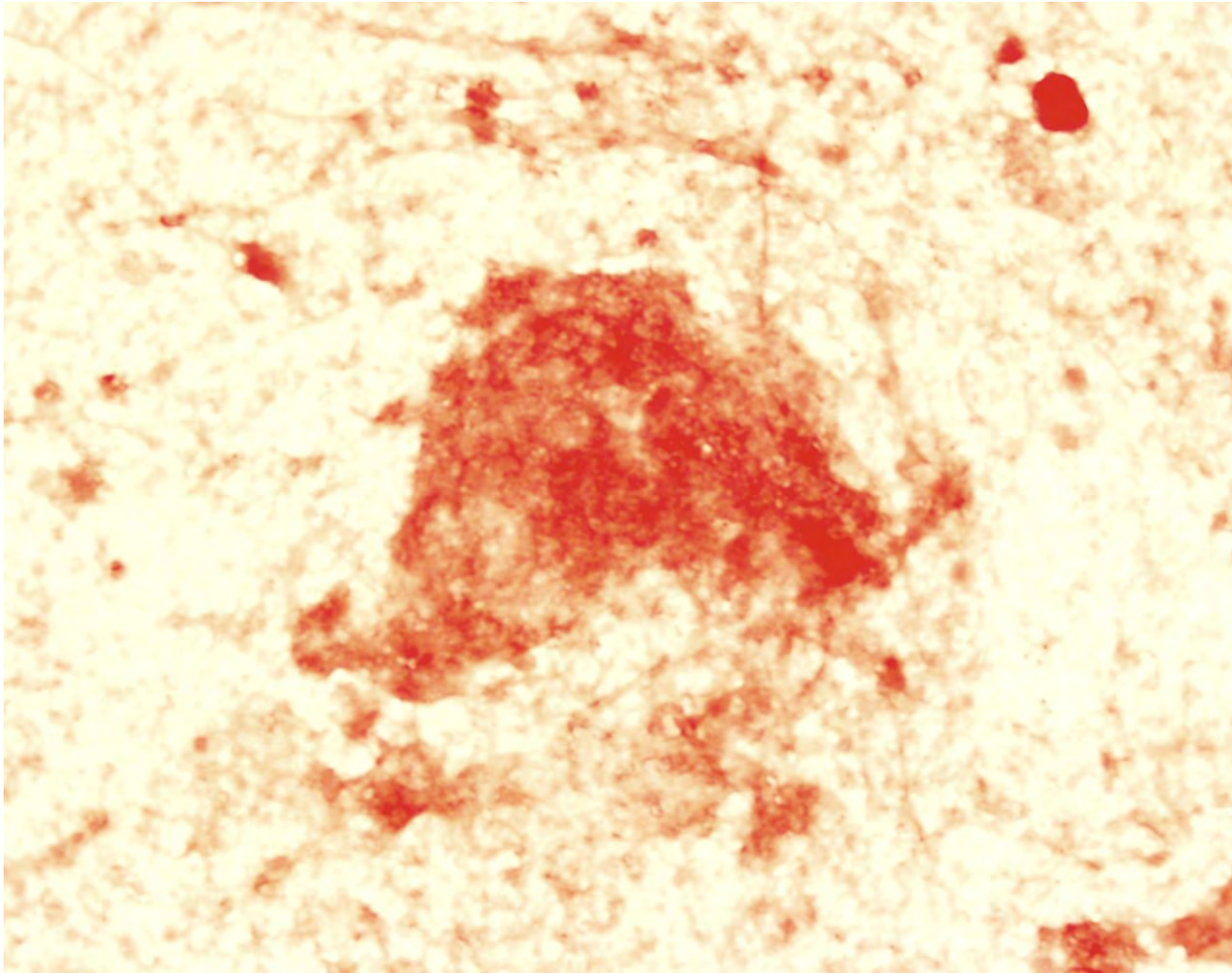
Gram stain x 1000

(Høiby 2009)

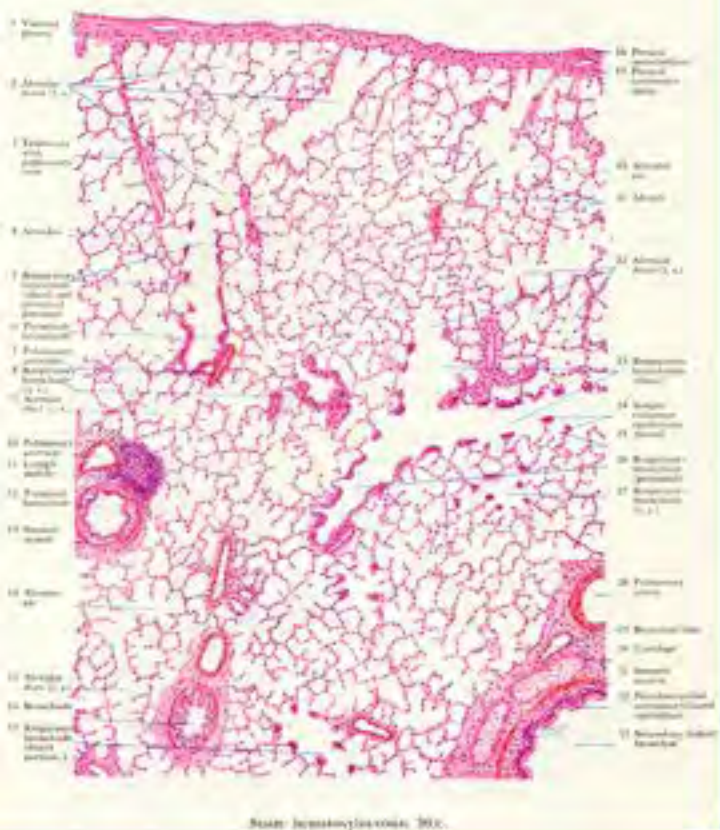
Biofilm in Sputum from two CF patients with chronic *Stenotrophomonas maltophilia* lung infection. Few PMNs



Gram stained smears x 1000



Achromobacter xylosoxidans biofilm in sputum from a chronically infected 23 year old CF patient (CF 370). Gram stain x 1000.

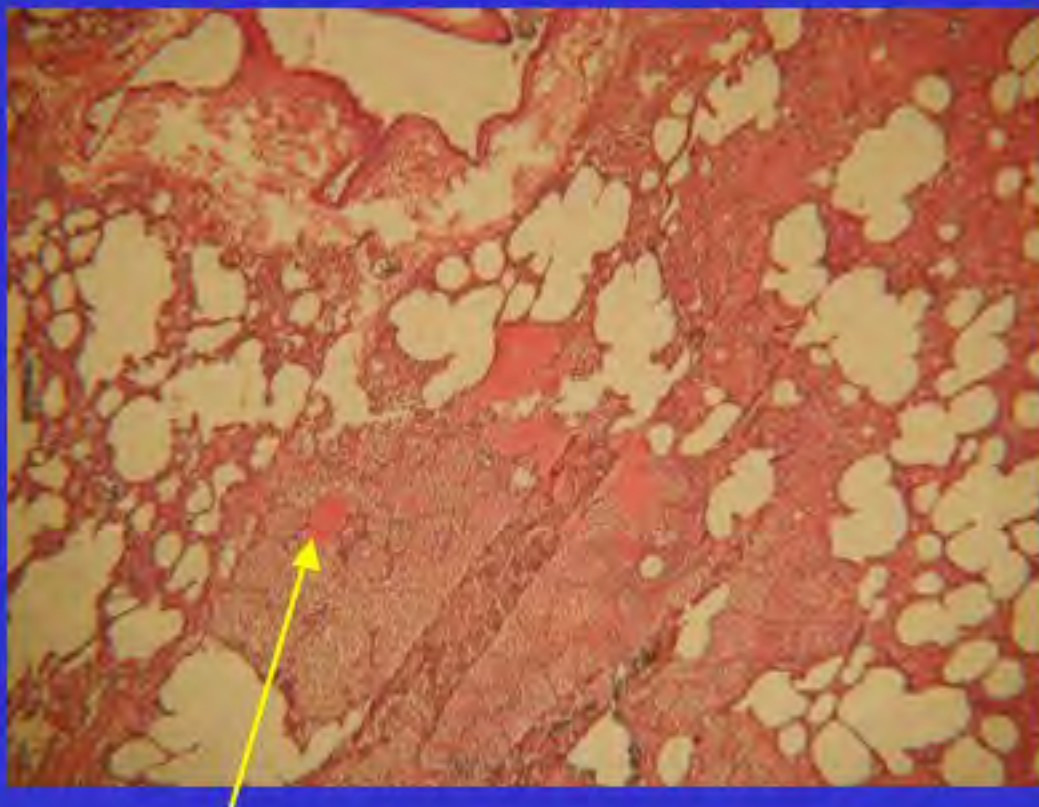


Stain: hematoxylin-eosin, 30X.

Normal lung

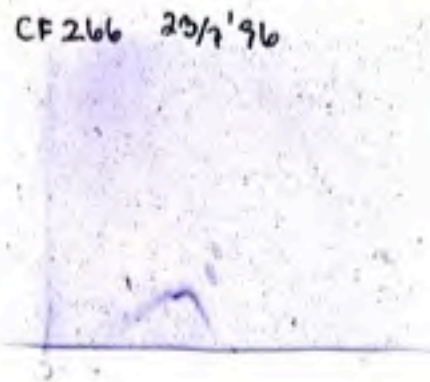
HØIBY 2004

The *packed* aerobic CF alveolar incubation chamber filled with mucoid *P. aeruginosa* biofilm

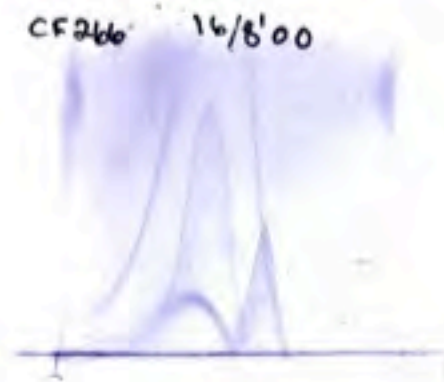
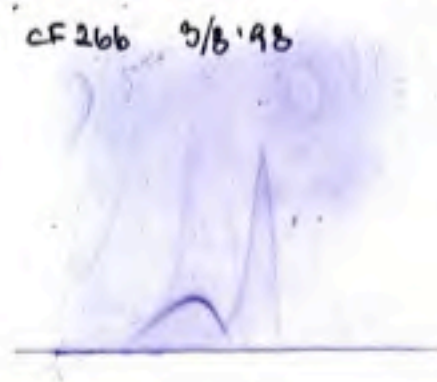


Autopsy (BS242/74) of a Danish CF girl (MLM) who died due to chronic *P. aeruginosa* lung infection and 21 precipitating antibodies against *P. aeruginosa* (normal: 0-1). Severely inflamed tissue (pneumonia). HE stain x 40.

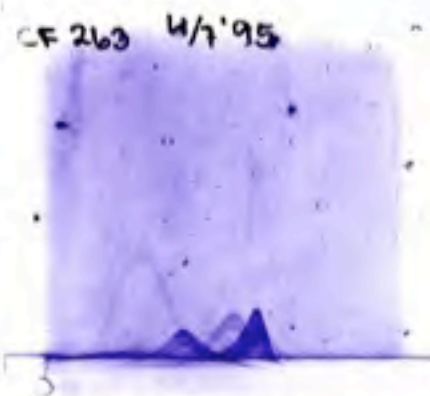
Antibodies against *P. aeruginosa* measured by crossed immunoelectrophoresis (CIE)



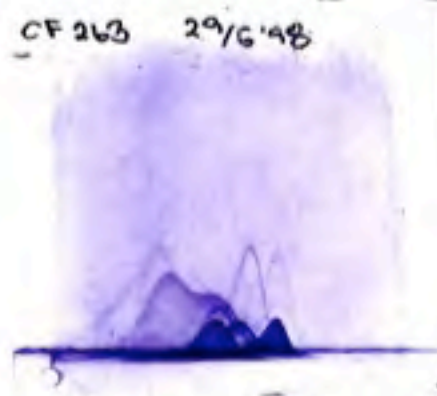
Slow increase



Antibodies – helpfull or harmfull?



Rapid increase



Normal: 0-1 precipitin



Susceptibility testing of planktonic growing bacteria does not reflect the susceptibility of the same bacteria growing as a biofilm!

Randomized Trial of Biofilm Testing to Select Antibiotics for Cystic Fibrosis Airway Infection

Samuel M. Moskowitz, MD,^{1,2,3*} Julia C. Emerson, MD, MPH,^{2,3} Sharon McNamara, MN,³
Richard D. Shell, MD,⁴ David M. Orenstein, MD,⁵ Daniel Rosenbluth, MD,⁶ Marcia F. Katz, MD,⁷
Richard Ahrens, MD,⁸ Douglas Hornick, MD,⁹ Patricia M. Joseph, MD,¹⁰ Ronald L. Gibson, MD, PhD,^{2,3}
Moirá L. Aitken, MD,¹¹ Wade W. Benton, PharmD and,³ Jane L. Burns, MD^{2,3}

Summary. Rationale: In cystic fibrosis (CF), conventional antibiotic susceptibility results correlate poorly with clinical outcomes. We hypothesized that biofilm testing would more accurately reflect the susceptibilities of bacteria infecting CF airways. Methods: A multicenter randomized pilot trial was conducted to assess the efficacy and safety of using biofilm susceptibility testing of *Pseudomonas aeruginosa* sputum isolates to guide antibiotic regimens for chronic airway infections in clinically stable adolescent and adult CF patients. Thirty-nine participants were randomized to biofilm or conventional treatment groups; 14-day courses of two antibiotics were selected according to an activity-based algorithm using the corresponding susceptibility results. Results: Of the agents tested, meropenem was most active against biofilm-grown bacteria, and was included in regimens for about half of each study group. For 19 of 39 randomized participants, randomization to the other study group would not have changed the antibiotic classes of the assigned regimen. Study groups were comparable at baseline, and had similar mean decreases in bacterial density, measured in log₁₀ colony forming units per gram of sputum (biofilm, −2.94 [SD 2.83] vs. conventional, −3.27 [SD 3.09]), and mean increases in forced expiratory volume in 1 sec, measured in liters (0.18 [SD 0.20] vs. 0.12 [SD 0.22]). Conclusions: In this pilot study, antibiotic regimens based on biofilm testing did not differ significantly from regimens based on conventional testing in terms of microbiological and clinical responses. The predictive value of biofilm testing may nonetheless warrant evaluation in an adequately powered clinical trial in younger CF patients or those experiencing acute pulmonary exacerbation. **Pediatr Pulmonol.** 2011; 46:184–192. © 2011 Wiley-Liss, Inc.

Key words: *Pseudomonas aeruginosa*; intravenous antibiotics; antibiotic resistance; antibiotic susceptibility testing; broth microdilution testing; inhibitory quotient; sputum bacterial density; lung function.

Comparison of biofilm and planktonic susceptibility testing of CF *P. aeruginosa* isolates

Antibiotic 90 strains	MIC ₅₀ (μ g/ml) (range)	BIC ₅₀ (μ g/ml) (range)
Piperacillin/Tz	4 (<1-1024)	256 >512
Ceftazidime	2 (<0.5-512)	128 (<2->128)
Aztreonam	4 (<2-64)	>128 (<2->128)
Meropenem	<1 (<1-16)	4 (<1->64)

(Moskowitz, S., J. Clin. Microbiol. 2004, 42:1915-22)

Oana Ciofu 2005

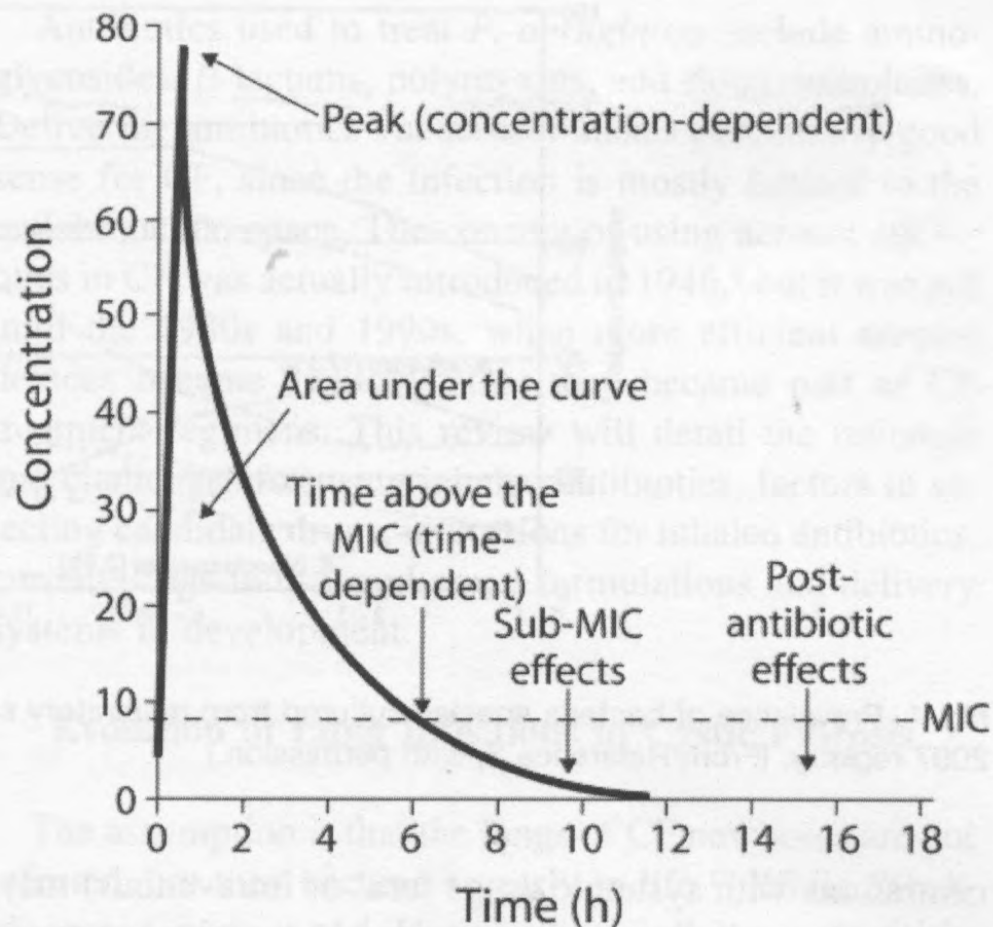


Fig. 3. Antibiotic pharmacodynamics. The graph is a representative concentration-versus-time curve of an antibiotic in the compartment of interest (in this case, the lungs). After inhalation, the level is very high, then falls due to absorption into the bloodstream or elimination through airway clearance. β -lactam antibiotics demonstrate time-dependent killing (the longer the time above the minimum inhibitory concentration [MIC] of the bacteria, the better). Aminoglycosides and fluoroquinolones demonstrate concentration-dependent killing (a high ratio of average maximum serum concentration to MIC or area-under-the-curve [AUC] to MIC work best).

Summary

Minimal bactericidal concentration (MBC)

minimal biofilm eradication concentration (MBEC)

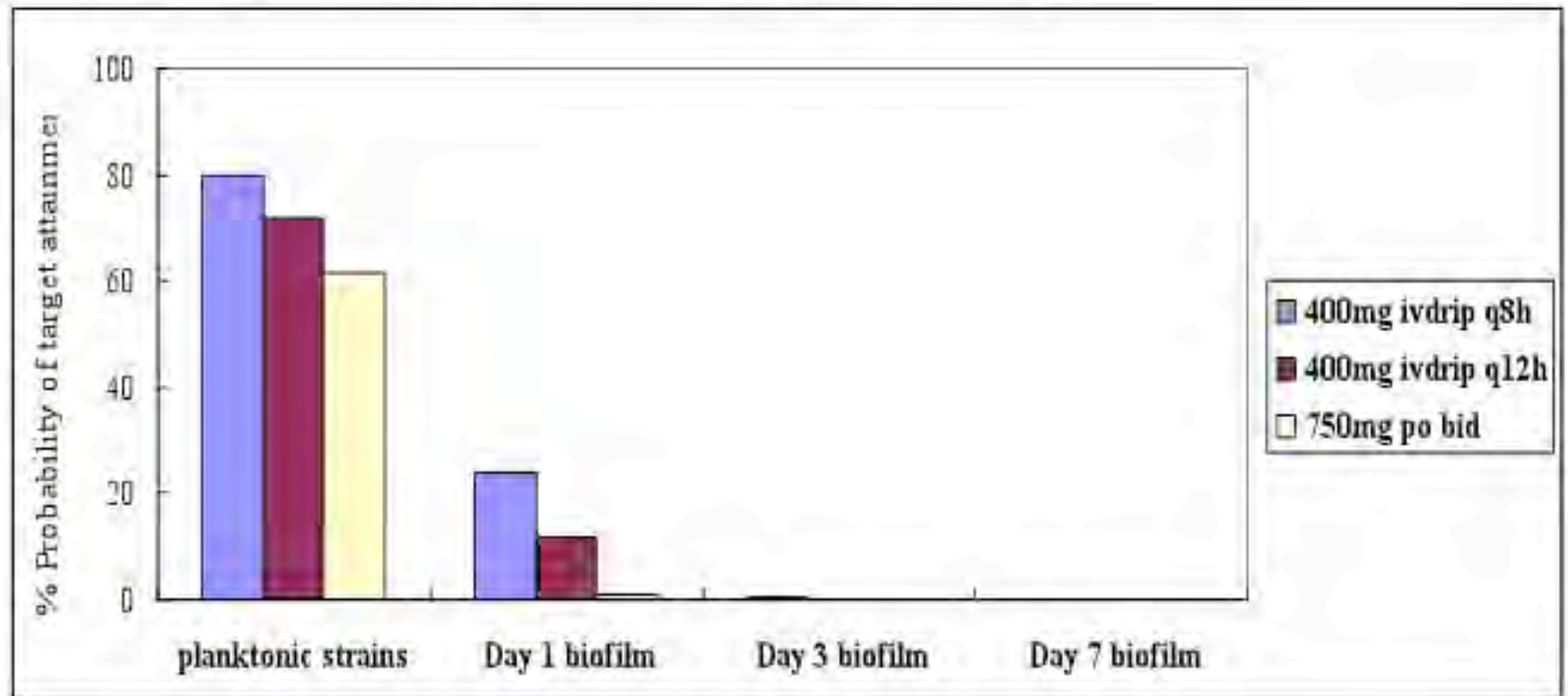
Minimal biofilm inhibition concentration (MBIC)

MBC (planktonic) & MBEC (biofilm) ($\mu\text{g/ml}$)

	Ciprofloxacin	Imipenem
Planktonic	2	8
Day 1 biofilm (non-mucoid PAO1)	8	256
Day 1 biofilm (Mucoid PDO300)	32	>256
Day 3 biofilm	32	>2048
Alginate beads	64	>256
PK/PD index	fC_{\max}/MIC (planktonic) $f\text{AUC}/\text{MIC}$ (planktonic) fC_{\max}/MBIC (biofilm) $f\text{AUC}/\text{MIC}$ (biofilm)	$fT > \text{MIC}$ (planktonic) $fT > \text{MBIC}$ (biofilm)

Ciprofloxacin Population Pharmacokinetics / Monte Carlo Simulation on CF patients on the basis of published data

✧ **Target: $fAUC_{0-24}/MIC$ ratio ≥ 125 (mg · h / liter)**



(Alan Forrest, AAC, 1993; Pedersen S. S, JAC, 1987; Montgomery M. J, AAC, 2001)

(Wang, Song, Wu & Høiby 2010)

Biofilm Infections - Prevention & Therapy

- Prophylaxis: 1) antibiotic prophylaxis, 2) Early aggressive antibiotic therapy when infection is suspected hopefully before the biofilm is fully mature, 3) Antibiotic- coated or silver-coated or antiseptic-solution-coated foreign body
- Therapy: Biofilm infections cannot be eradicated by antibiotic therapy. The strategy is therefore either to replace the foreign body (if possible) covered by antibiotic therapy, and preferably after ≥ 24 h interval to insert a new, clean foreign body using "prolonged" pathogen-directed antibiotic prophylaxis for 14 days (until endothelial/epithelial growth and coverage of the surface/inside place is finished. If that cannot be done, then the strategy is to use continuous suppression of the biofilm growth by giving the patient pathogen directed antibiotic treatment for prolonged time, in some cases (e.g. aortic grafts) for the rest of the life of the patient

I WILL BE HAPPY TO ANSWER ANY QUESTION.....



**.....FROM THE
AUDIENCE.....**

